Available online at www.sciencedirect.com
ScienceDirect
Veterinary Immunology and Immunopathology 121 (2008) 370–377
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Short communication

Channel catfish, *Ictalurus punctatus*, cyclophilin A and B cDNA characterization and expression analysis

Hung-Yueh Yeh *, Phillip H. Klesius

United States Department of Agriculture, Agricultural Research Service, Aquatic Animal Health Research Unit, 990 Wire Road, Auburn, AL 36832, USA

Received 17 August 2007; received in revised form 10 September 2007; accepted 26 September 2007

Abstract

The preliminary observation of up-regulation of cyclophilin transcripts during *Edwardsiella ictaluri* infection prompted us to speculate on the potential importance of cyclophilins in the early stage of infection. To provide a framework for answering these questions, two cyclophilin cDNA of channel catfish, *Ictalurus punctatus*, were identified, sequenced and characterized. The complete nucleotide sequences of cyclophilin A and cyclophilin B cDNA consisted of 1170 and 996 bases, respectively. Analyses of the sequences revealed each had one open reading frame potentially encoding 164 amino acids with calculated molecular mass of 17,450 Da and 216 amino acids with calculated molecular mass of 23,852 Da for cyclophilin A and cyclophilin B, respectively. The degrees of conservation of channel catfish cyclophilin A and cyclophilin B amino acid sequences to counterparts of other species ranged from 74 to 84% and 80 to 92%, respectively. Both cyclophilin A and cyclophilin B transcripts were constitutively expressed in all tissues of channel catfish examined in this study. These results provide valuable information not only for further exploring the roles of cyclophilins in fish immune responses to infection, but also for production of polyclonal/monoclonal antibodies for channel catfish cyclophilins.
Published by Elsevier B.V.

Keywords: Cyclophilin; Peptidyl-prolyl isomerase; Channel catfish; *Ictalurus punctatus*

1. Introduction

Cyclophilin A (CyPA) and cyclophilin B (CyPB) are intracellular members of the highly conserved immunophilin family and are ubiquitous (Bukrinsky, 2002; Galat, 1999; Ivery, 2000; Pemberton, 2006; Maruyama and Furuani, 2000; Wang and Heitman, 2005; He et al., 2004). Both proteins play many important roles in physiological and pathological processes. In physiological conditions, both proteins possess peptidyl-prolyl *cis–trans* isomerase activity that mediates protein folding as well as multidomain protein assembly, followed by serving as signal molecules (Wang and Heitman, 2005; Brazin et al., 2002; Min et al., 2005). In addition, Bukrinsky and other groups demonstrated that CyPA and CyPB use CD147, a widely expressed membrane protein, as a signal receptor resulting in chemotaxis and adhesion to the extracellular matrix, respectively (Allain et al., 2002; Yurchenko et al., 2001, 2002, 2005, 2006). CyPA binds strongly to the immunosuppressive agent, cyclosporine A, to form a complex that subsequently blocks T cell activation (Schmid, 2001; Heitman et al., 1992) via Itk tyrosine kinase (Brazin et al., 2002; Colgan et al., 2004). Another study demonstrated that CyPA interacts with peroxiredoxins and subsequently activates their peroxidase activity (Lee et al., 2001). A study has shown that CyPB and interferon regulatory factor-3 interaction

* Corresponding author. Tel.: +1 334 887 3741; fax: +1 334 887 2983.
E-mail address: yyeh@ars.usda.gov (H.-Y. Yeh).

0165-2427/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetimm.2007.09.015

resulted in suppression of phosphorylation of interferon regulatory factor-3 and thereby inhibits interferon-β induction (Obata et al., 2005).

On the other hand, cyclophilins have been implicated in microbial pathogenesis. Interactions of CyPA with various human immunodeficiency virus-1 proteins are required for establishment of infection in human cells (e.g. Luban, 2007; Billich et al., 1995; Zander et al., 2003). CyPB interacts with the hepatitis C virus RNA polymerase NS5B and then promotes the replication of the viral genome (Watashi et al., 2005). In the course of studying *Edwardsiella ictaluri* pathogenesis, up-regulation of cyclophilin expressed sequence tags (EST) during *Ed. ictaluri* infection in channel catfish (CC) ovary cell (ATCC CRL-2772) line was observed (Yeh and Klesius, unpublished observation). This preliminary observation prompted us to speculate that these cyclophilins may play a critical role in the early stage of *Ed. ictaluri* infection. In order to provide a framework for answering these questions, CC CyPA and CyPB were isolated, characterized and expressed. Both cyclophilins were constitutively expressed in all tissues analyzed.

2. Materials and methods

2.1. Animals

Channel catfish (NWAC 103 strain, 20–25 g) were maintained at the Aquatic Animal Health Research Unit, Agricultural Research Service, United States Department of Agriculture, and were acclimated for 2 weeks at 28 °C before use in experiments (Jenkins and Klesius, 1998). All fish were clinically healthy. The protocol for animal usage in experiments was approved by the Institutional Animal Care and Use Committee, Aquatic Animal Health Research Unit, Agricultural Research Service, United States Department of Agriculture.

2.2. Tissue samples

Fish were euthanized by immersion in tricaine methanesulfonate (MS-222) according to the Guidelines for the Use of Fishes in Research (Nickum et al., 2004). Gills, skin, spleen, hepatopancreas, intestine and head kidneys were aseptically excised.

2.3. RNA isolation and construction of CC rapid amplification of cDNA ends (RACE)

Total RNA from the excised tissues was extracted by using a Tri reagent kit (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's protocol. The quality and quantity of total RNA was determined by an Agilent Bioanalyzer using RNA 1200 chips (Agilent Technologies, Santa Clara, CA). Both 16S and 28S RNAs were clearly identified.

For RACE library construction, a GeneRacer kit (Invitrogen Corp., Carlsbad, CA) was used according to the manufacturer's protocol. Both 5′- and 3′-RACE cDNAs were amplified by PCR. The primers synthesized by Sigma-Genosys (The Woodlands, TX) for PCR amplification are listed in Table 1. The PCR products were purified by agarose gel electrophoresis and ligated into a pCR4-TOPO TA vector (Invitrogen Corp.). The ligated plasmids were transformed into TOP10 *E. coli* by heat-shock. After enrichment in the S.O.C. medium, cells were streaked on LB plates containing 50 μg/ml of ampicillin and incubated at 35 °C overnight. Colonies were randomly picked and cultivated in WU medium for sequencing.

Table 1
Oligonucleotides used in this study

Primer	Sequence	Tm (°C)
GeneRacer 5′Primer (Invitrogen)	5′-CGACTGGAGCACGAGGACACTGA-3′	74
GeneRacer 3′Primer (Invitrogen)	5′-GCTGTCAACGATACGCTACGTAACG-3′	78
β-Actin F	5′-GACTTCGAGCAGGAGATGGG-3′	72
β-Actin R	5′-AACCTCTCATTGCCAATGGTG-3′	69
(1) Cyclophilin A[a]		
CPA38F	5′-GCAGCGGCAAGTGCTCTGCCAAGATA-3′	78
CPA42R	5′-GCTGCTGCTTCCTCTGCCTTCGACAA-3′	77
CPA207R	5′-ACCCTGGCACATGAAACCTGGGATGA-3′	77
(2) Cyclophilin B[a]		
CPB116F	5′-TCATGATCCAAGGAGGCGACTTCACCA-3′	77
CPB366F	5′-AGGCACAAAAACGGACGGCAGGGATA-3′	77
CPB299R	5′-CATGGCGTCTGCACGGTGGTAATGAA-3′	77
CPB385R	5′-TGCCGTCCGTTTTTGTGCCTTCAATC-3′	77

[a] Sequences used for cyclophilin A and B primer design were from DQ086168 and DQ086177, respectively.

2.4. DNA sequencing and analyses

DNA sequencing on both strands was carried out at the USDA ARS MidSouth Genomic Laboratory (Stoneville, MS) with an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequencing chromatograms were edited for quality and trimmed vector sequence using the Phred (Ewing and Green, 1998; Ewing et al., 1998) and Lucy 2 (Li and Chou, 2004) programs. The CC cyclophilin amino acid sequences were deduced from nucleic acid sequences by using the Transeq program via the Internet (Rice et al., 2000), and aligned with other cyclophilin amino acid sequences by using the ClustalW (version 1.83) software (Chenna et al., 2003). The phylogenetic analyses were performed by using the MEGA (version 3.1) software (Kumar et al., 2004) based on the ClustalW alignment result. The AliBaba2.1 software was used to analyze the promoters (Grabe, 2002).

2.5. RT-PCR

RT-PCR assays for cyclophilin gene expression in CC tissues were carried out by a two-step procedure routinely used in our laboratory (Yeh and Klesius, 2007a,b). First, total RNA from various tissues were reverse transcribed into cDNA by a Superscript reverse transcriptase (Invitrogen Corp.) in the presence of random hexamers. For PCR, 50 μl reactions contained (in final concentrations) 1× PCR buffer (TaKaRa Mirus Bio, Madison, WI), 3.0 mM $MgCl_2$ (Applied Biosystems), 200 μM dNTP (TaKaRa Mirus Bio), 0.3 μM each of gene-specific primers (Table 1), 1.25 U *ExTaq* HS DNA polymerase (TaKaRa Mirus Bio) and cDNA template. The amplification was performed initially at 94 °C for 2 min, followed by 25 cycles of 94 °C for 15 s, 62 °C for 1 min, 72 °C for 2 min. The final extension was carried out at 72 °C for 10 min. The reaction mixture without cDNA template served as a negative control. The amplified PCR fragments were analyzed in 2% agarose gel electrophoresis and stained with ethidium bromide. Images were documented by a KODAK Gel Logic 440 Imaging System and processed by an Adobe Photoshop program (v. 7.0.1).

3. Results and discussion

3.1. Cloning and characterization of CC cyclophilin cDNA

In the preliminary study, the subtractive suppression hybridization was used to partially analyze cDNA libraries from CC ovary cell line (ATCC CRL-2772), and we found that several clones contained cyclophilin EST (GenBank Accession Nos. DQ086168 and DQ086177). Based on these EST, these genes were further cloned and sequenced by using the rapid amplification of cDNA ends (RACE) method (Frohman et al., 1988). The complete sequence of the CC CyPA cDNA consisted of 1170 nucleotides. Analysis of the nucleotide sequence shows one open reading frame (ORF) and 5′- and 3′-end untranslated regions (UTR) (data not shown, GenBank Accession No. EF424274). The 5′-UTR had 104 bases containing a potential TATTA box (74–78 bases) and a Sp1 transcription factor binding site (29–38 bases). The 3′-UTR was 571 bases in length. Like mammalian cDNA, the CC CyPA cDNA contained a potential mRNA instability motif (741–746 bases) that regulates mRNA degradation (Zubiaga et al., 1995), two polyadenylation signal sequences (1097–1102 and 1101–1106 bases), and a polyadenylation tail. The ORF potentially encoded a 164-amino acid peptide with a calculated molecular weight of 17,450 Da.

The complete sequence of the CC CyPB cDNA gene consisted of 996 nucleotides. The 5′-UTR had 77 bases including two Sp1 transcription factor binding sites (13–23 and 46–55 bases), while the 3′-UTR had 268 bases containing a polyadenylation signal sequence (944–949 bases) and a 29-base polyadenylation tail (data not shown, GenBank Accession No. EF424275). The ORF potentially encoded a 216-amino acid peptide with a calculated molecular mass of 23,852 Da.

3.2. Alignment and phylogenetical analysis of CC cyclophilin amino acid sequences

To determine the similarity and evolutionary relatedness of CC cyclophilin amino acid sequences with other known cyclophilin sequences deposited in GenBank, the ClustalW (Chenna et al., 2003) and MEGA3 (Kumar et al., 2004) software programs were used to analyze the cyclophilin sequences (Figs. 1 and 2). CyPA amino acid sequences were highly conserved among species, ranging from 74% (CC vs. Chinese hamster) to 84% (CC vs. zebrafish) (Fig. 1A). Two important features for CyPA functions were conserved in CC. First, based on a structural study of human cyclophilin A (Ke et al., 1991), CC CyPA protein potentially has four β-strands and a loop (Lys118 to His126) indicating that it could form a pocket for the cyclosporine A binding. In addition, CC CyPA conserved the amino acid residues (His54, Arg55, Phe60, Phe113 and His126) that involve peptidyl-prolyl

(A)

```
                                                                    <-
Mouse                   MVNPTVFFDITADDEPLGRVSFELFADKVPKTAENFRALSTGEKGFGYKG 50
Chinese hamster         MVNPTVFFDISADGEPLGRVSFELFADKVPKTAENFRALSTGEKGFGYKG 50
Norway rat              MVNPTVFFDITADGEPLGRVCFELFADKVPKTAENFRALSTGEKGFGYKG 50
Human                   MVNPTVFFDIAVDGEPLGRVSFELFADKVPKTAENFRALSTGEKGFGYKG 50
Rabbit                  MVNPTVFFDIAVDGEPLGRVSFELFADKVPKTAENFRALSTGEKGFGYKG 50
Chlamys farreri         MSNPRVFFDVTANGKPVGRIMMELRGDVVPKTAENFRALCTGEKGFGYKG 50
Sea urchin              MAKPQVFFDLQANGENLGRIVMELRADVVPKTAENFRALCTGEKGFGYKG 50
Channel catfish         MSKPKVFFDITIDGKSAGRIVIELRADVVPKTAENFRALCTGEKGFGYKG 50
Zebrafish               MANPKVFFDITIDGKEAGRIVMELRADVVPKTAENFRALCTGEKGFGYKG 50
Pacific oyster          MGKPQVFFDISIGGQPAGRIVMELEADVVPKTAENFRALCTGEKGFGYKG 50
Tetraodon nigroviridis  MANPRVFFDIAINGKPAGRIVMELNADVVPKTAENFRALCTGEKGFGYKG 50
                        *  * ****        **   **  * *********** **********
                         β3 ->   <-β4->                                <-β5-
Mouse                   SSFHRIIPGFMCQGGDFTRHNGTGGKSIYGEKFEDENFILKHTGPGILSM 100
Chinese hamster         SSFHRIIPGFMCQGGDFTRHNGTGGRSIYGEKFEDENFILKHTGPGILSM 100
Norway rat              SSFHRIIPGFMCQGGDFTRHNGTGGKSIYGEKFEDENFILKHTGPGILSM 100
Human                   SCFHRIIPGFMCQGGDFTRHNGTGGKSIYGEKFEDENFTLKHTGPGILSM 100
Rabbit                  SCFHRIIPGFMCQGGDFTRHNGTGGKSIYGEKFEDENFLLKHTGPGILSM 100
Chlamys farreri         SPFHRVIPSFMCQGGDFTRQNGTGGKSIYGEKFADENFTLKHTGPGVLSM 100
Sea urchin              STFHRVIPGFMCQGGDFTRHNGTGGKSIYGEKFADENFTLKHTQPGILSM 100
Channel catfish         SGFHRVIPGFMCQGGDFTNHNGTGGKSIYGNKFADENFTLKHTGPGIMSM 100
Zebrafish               SGFHRVIPQFMCQGGDFTNHNGTGGKSIYGNKFEDENFTLKHGGKGTLSM 100
Pacific oyster          SGFHRVIPQFMCQGGDFTNHNGTGGKSIYGNKFPDENFQLKPLGPGTLSM 100
Tetraodon nigroviridis  STFHRVIPQFMCQGGDFTNHNGTGGKSIYGNKFQDENFSLTHTGPGILSM 100
                        * *** ** *********  ***** **** ** **** *     *  **
                        >         <-β6-> <-Loop->
Mouse                   ANAGPNTNGSQFFICTAKTEWLDGKHVVFGKVKEGMNIVEAMERFGSRNG 150
Chinese hamster         ANAGPNTNGSQFFICTAKTEWLDGKHVVFGKVKEGMNIVEAMERFGSRNG 150
Norway rat              ANAGPNTNGSQFFICTAKTEWLDGKHVVFGKVKEGMSIVEAMERFGSRNG 150
Human                   ANAGPNTNGSQFFICTAKTEWLDGKHVVFGKVKEGMNIVEAMERFGSRNG 150
Rabbit                  ANAGPNTNGSQFFICTAKTEWLDGKHVVFGRVKEGMSIVEAMEHFGSENG 150
Chlamys farreri         ANAGPNTNGSQFFLCTARTEWLDGKHVVFGSVVEGMDVVKKIESYGSQSG 150
Sea urchin              ANAGVNTNGSQFFICTAVTSWLDGKHVVFGAVTQGLDIIKKVESYGSDSG 150
Channel catfish         ANAGPNTNGSPFFICTEKTSWLDGKHVVFGSVVDGMDVVRVVEGRGSSSG 150
Zebrafish               ANAGPNTNGSQFFICTADTNWLDGKHVVFGKVVDGLNVVDAIEKKGSSSG 150
Pacific oyster          ANAGPNTNGSQFFICTEKTSWLDGKHVVFGRVTDGMNVVKAMEATGSQSG 150
Tetraodon nigroviridis  ANAGPNTNGSQFFICTVKTQWLDGKHVVFGKVVEGMDLVQTMESYGSQSG 150
                        **** ***** ** **  * ********** *  *       *  **  *

Mouse                   KTSKKITISDCGQL- 164
Chinese hamster         KTSKKITISDCGQL- 164
Norway rat              KTSKKITISDCGQL- 164
Human                   KTSKKITIADCGQLE 165
Rabbit                  KTSKKITIANCGQL- 164
Chlamys farreri         KTSEKIIVADCGQL- 164
Sea urchin              KTSKKITIADCGQL- 164
Channel catfish         KCFAKIVIADCGQL- 164
Zebrafish               KCSAKVVIANCGQL- 164
Pacific oyster          KPSKPIKIENCGQL- 164
Tetraodon nigroviridis  KPKANITIADCGQL- 164
                        *         ****
```

Fig. 1. Multiple alignments of the predicted channel catfish cyclophilin amino acid sequences with those from other species. (A) Cyclophilin A: the cyclosporine A binding domains (four β-strands and one loop) are indicated on the top of sequences (Ke et al., 1991). Species and corresponding Accession No. used for alignment are as follows: mouse (BAE30323), Chinese hamster (P14851), Norway rat (NP_058797), human (AAH05982), rabbit (Q9TTC6), *Chlamys farreri* (AAR11779), sea urchin (P91791), zebrafish (AAQ91263), Pacific oyster (AAT44353) and *Tetraodon nigroviridis* (CAF94597). (B) Cyclophilin B: the cyclosporine A binding domains are indicated on the top of sequences. In addition, the glycosaminoglycan binding sites are in bold. Species and corresponding Accession No. used for alignment are following: mouse (NP_035279), rat (AAH61791), human (NP_000933), chimpanzee (XP_001174161), cattle (AAX08983), dog (XP_852389), chicken (NP_990792), African clawed frog (AAH54168), zebrafish (AAQ91263) and *T. nigroviridis* (CAF98384). Identical amino acids among all sequences are indicated with asterisks.

(B)

```
                                ←              Domain I                 →←
Mouse                   MLRLSERNMKVLFAAALIVGSVVFLLLPGPSVANDKKKGPKVTVKVYFDL 50
Rat                     MLRLSERNMKVLFAAALIVGSVVFLLLPGPSVANDKKKGPKVTVKVYFDF 50
Human                   MLRLSERNMKVLLAAALIAGSVFFLLLPGPSAADEKKKGPKVTVKVYFDL 50
Chimpanzee              MLRLSERNMKVLLAAALIAGSVFFLLLPGPSVADEKKKGPKVTVKVYFDL 50
Cattle                  MLRLSERNMKILFVAALVVGSVFFLLLPGPSAADEKKKGPKVTVKVYFDL 50
Dog                     MLRLSERNMKVLFAAALVVGSVFFLLLPGPSTADEKKKGPKVTVKVYFDL 50
Chicken                 --------MKALVAAT-ALGPALLLLLPAASRADERKKGPKVTAKVFFDL 41
African clawed frog     MLRPLERNMKLLFAAALIAGSVIFLLFPGSSVADDKKKGPKVTDKVYFDL 50
Channel catfish         MVRACERKMKFLVAVTIIVASVFFLLLPNGSQADEKKKGPKVTAKVYFDI 50
Zebrafish               MVRICERRMKFLVAVTLIVGSVVFLLFPSETEADEKKKGPKVTAKVYFDI 50
Tetraodon nigroviridis  MLRLFGRRMKVLVAVTVIVGSLIFLAFPNGSSADDKKRGPKVTAKVYFDM 50
                                ** *           *  *    *   * ***** ** **
                                   Domain II
Mouse                   QIGDESVGRVVFGLFGKTVPKTVDNFVALATGEKGFGYKNSKFHRVIKDF 100
Rat                     QIGDEPVGRVTFGLFGKTVPKTVDNFVALATGEKGFGYKNSKFHRVIKDF 100
Human                   RIGDEDVGRVIFGLFGKTVPKTVDNFVALATGEKGFGYKNSKFHRVIKDF 100
Chimpanzee              RIGDEDVGRVIFGLFGKTVPKTVDNFVALATGEKGFGYKNSKFHRVIKDF 100
Cattle                  RIGDEDIGRVVIGLFGKTVPKTVDNFVALATGEKGFGYKDSKFHRVIKDF 100
Dog                     RIGDEDIGRVVIGLFGKTVPKTVDNFVALATGEKGFGYKDSKFHRVIKDF 100
Chicken                 RVGEEDAGRVVIGLFGKTVPKTVENFVALATGEKGFGFKGSKFHRVIKDF 91
African clawed frog     KIGDEEVGRIVIGLFGKTVPKTVENFVTLATGEKGYGYKGSKFHRVIKEF 100
Channel catfish         RVGDEDIGRIVIGLFGKTVPKTTENFLALATGEKGFGYKGSKFHRVIKDF 100
Zebrafish               KIGDEDAGRIVIGLFGKTVPKTTENFLQLATGEKGFGYKGSKFHRVIKDF 100
Tetraodon nigroviridis  KIGDEEIGRIVIGLFGKTVPKTVDNFVALATGEKGFGYKGSKFHRVISQF 100
                          * *  **   **********  **  ******* * * *******  *
                        →←                        Domain III
Mouse                   MIQGGDFTRGDGTGGKSIYGERFPDENFKLKHYGPGWVSMANAGKDTNGS 150
Rat                     MIQGGDFTRGDGTGGKSIYGERFPDENFKLKHYGPGWVSMANAGKDTNGS 150
Human                   MIQGGDFTRGDGTGGKSIYGERFPDENFKLKHYGPGWVSMANAGKDTNGS 150
Chimpanzee              MIQGGDFTRGDGTGGKSIYGERFPDENFKLKHYGPGWVSMANAGKDTNGS 150
Cattle                  MIQGGDFTRGDGTGGKSIYGERFPDENFKLKHYGPGWVSMANAGKDTNGS 150
Dog                     MIQGGDFTRGDGTGGKSIYGERFPDENFKLKHYGPGWVSMANAGKDTNGS 150
Chicken                 MIQGGDFTRGDGTGGKSIYGDRFPDENFKLKHYGPGWVSMANAGKDTNGS 141
African clawed frog     MIQGGDFTRGDGTGGKSIYGDRFPDENFKLKHYGPFWLSMANAGKDTNGS 150
Channel catfish         MIQGGDFTRGDGTGGKSIYGDRFPDENFKLKHYGPGWLSMANAGKDTNGS 150
Zebrafish               MIQGGDFTRGDGTGGKSIYGDRFPDENFKLKHYGPGWLSMANAGKDTNGS 150
Tetraodon nigroviridis  MIQGGDFTRGDGTGGKSIYGDRFPDENFKLKHYGPGWLSMANAGKDTNGS 150
                        ******************** ************** * ************
                                              →←      Domain IV
Mouse                   QFFITTVKTSWLDGKHVVFGKVLEGMDVVRKVESTKTDSRDKPLKDVIIV 200
Rat                     QFFITTVKTSWLDGKHVVFGKVLEGMDVVRKVENTKTDSRDKPLKDVIIV 200
Human                   QFFITTVKTAWLDGKHVVFGKVLEGMEVVRKVESTKTDSRDKPLKDVIIA 200
Chimpanzee              QFFITTVKTAWLDGKHVVFGKVLEGMEVVRKVESTKTDSRDKPLKDVIIA 200
Cattle                  QFFITTVKTAWLDGKHVVFGKVLEGMDVVRKVESTKTDGRDKPLKDVTIA 200
Dog                     QFFITTVKTAWLDGKHVVFGKVLEGMEVVRKVESTKTDSRDKPLKDVTIA 200
Chicken                 QFFITTVKTAWLDGKHVVFGKVLEGMDVVRKVENTKTDSRDKPLKDVTIA 191
African clawed frog     QFFITTVKTPWLDGKHVVFGKVLEGTEIVRKIESTKTDGRDKPLKDVVIA 200
Channel catfish         QFFITTVQTPWLDGKHVVFGKILEGMDVVRKIEGTKTDGRDKPLKDVIIH 200
Zebrafish               QFFITTVQTPWLDGKHVVFGKILEGMDVVRKIEATKTDGRDKPLKDVSIH 200
Tetraodon nigroviridis  QFFITTVTTTWLDGKHVVFGKVLEGMDVVLKIEKTKTDARDRPLKDVVIH 200
                        ******* * *********** ***   * * * **** ** ***** *
                            →←Domain V→
Mouse                   DSGKIEVEKPFAIAKE 216
Rat                     DCGKIEVEKPFAIAKE 216
Human                   DCGKIEVEKPFAIAKE 216
Chimpanzee              DCGKIEVEKPFAIAKE 216
Cattle                  DCGKIEVEKPFAIAKE 216
Dog                     DCGKIEVEKPFAIAKE 216
Chicken                 DCGTIEVEKPFAIAKE 207
African clawed frog     DCGKIEVEKPFAIAKE 216
Channel catfish         DSGKIEVEKPFAVAKE 216
Zebrafish               DSGKIDVEKPFAVAKE 216
Tetraodon nigroviridis  DSGVIEVEKPFAVPKE 216
                        * * * ******  **
```

Fig. 1. (*Continued*).

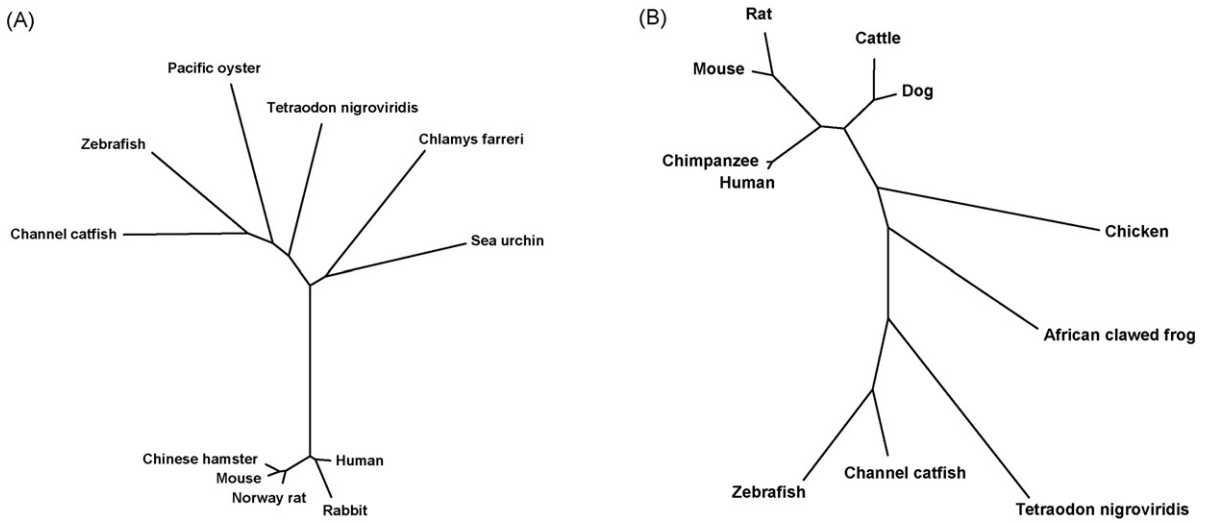

Fig. 2. Phylogenetic relationships of channel catfish cyclophilin A (A) and cyclophilin B (B) amino acids. Sequences from Fig. 1 were used to produce the phylogenetic tree by the Neighbor-Joining bootstrap analysis (500 replicates) in MEGA3 phylogenetic analysis program (Kumar et al., 2004).

isomerase activity (Zydowsky et al., 1992). Two recent reports identified the CyPA of common carp (*Cyprinus carpio*) and red stingray (*Dasyatis akajei*) (Kawano et al., 2003; Tu et al., 2003). Comparison of their amino acid sequences against those deposited in GenBank was made by using the BLASTp program (Altschul et al., 1997). We found that the common carp amino acid sequence had 85% identity to human cyclophilin-like 1 protein that is a 301-amino acid peptide with a molecular mass of 33 kDa (Kim et al., 1998; Mi et al., 1996), and the red stingray amino acid sequence had 75 and 77% identity to the human cyclophilin F (a 207-amino acid protein (Bergsma et al., 1991)) and douroucouli TRIM/CyPA fusion protein, respectively. As seen in Fig. 2A, two well-supported clades were formed. The tree also demonstrates that the CC CyPA was closely related to that of zebrafish.

Like CyPA, the CyPB amino acid sequences were highly conserved among species, ranging from 80% (CC vs. chicken) to 92% (CC vs. zebrafish) (Fig. 1B). The CC CyPB can be structurally divided into five domains (*I–V*) according to the study by Price et al. (1991). Domain I including a signal peptide (Bukrinsky, 2002) is the least conserved region among species and not found in the CC CyPA. Both the cyclosporine A binding sites in domains II and III and the glycosaminoglycan binding sites Lys36–Lys37–Lys38 and Tyr47–Phe48–Asp49) were well conserved in CC CyPB (Allain et al., 2002; Price et al., 1991). As seen in Fig. 2B, the evolutionary tree shows the phylogeny of the organism follows classical taxonomy and phylogenetic transition from solely water-living fish to amphibian frog to land inhabitants. The tree also demonstrates that the CC CyPB was close to that of zebrafish. However, we do not know at this time whether fish, a heterogeneous group of more than 23,000 species (Helfman et al., 1997) adapted to a wide range of environments, form well-supported clusters. In our recent studies, we observed a high degree of diversity of hemoglobin-β and CD59 among fish (Yeh et al., 2006; Yeh and Klesius, 2007b).

3.3. Expression of cyclophilins in CC tissues

RT-PCR amplification of mRNA from CC spleen, head kidney, liver, intestine, skin and gill was used to profile tissue expression of cyclophilins. Both CyPA and CyPB were expressed in all tissues of fish examined (data not shown). Individual variations were also observed. These results are in agreement with the notion that cyclophilins are ubiquitous in animal tissues (Koletsky et al., 1986; Ryffel et al., 1991).

In conclusion, CC cyclophilin cDNA were cloned and characterized. The degrees of conservation of CC CyPA and CyPB amino acid sequences to counterparts of other species ranged from 74 to 84% and 80 to 92%, respectively. Both CyPA and CyPB transcripts were constitutively expressed in all tissues examined. These results provide valuable information not only for further exploring the roles of cyclophilins in fish immune responses to infection, but also for production of polyclonal/monoclonal antibodies for CC cyclophilins.

The *in vitro* and *in vivo* roles of cyclophilin transcripts and their gene products on catfish infection with *Ed. ictaluri* are currently under investigation.

Acknowledgements

We thank Ms. Ifayet Johnson-Mayo and Ms. Ann Cooper of USDA ARS AAHRU for excellent technical support, Dr. Brian E. Scheffler and his team at the USDA ARS MidSouth Genomics Laboratory for sequencing and bioinformatics, and Drs. Kenneth E. Nusbaum (Auburn University, Auburn, AL) and Thomas L. Welker (AAHRU, ARS, USDA, Auburn, AL) for critical comments on the manuscript.

This study was supported by the CRIS Project No. 6420-32000-020-00D. Mention of trade names or commercial products in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References

Allain, F., Vanpouille, C., Carpentier, M., Slomianny, M.C., Durieux, S., Spik, G., 2002. Interaction with glycosaminoglycans is required for cyclophilin B to trigger integrin-mediated adhesion of peripheral blood T lymphocytes to extracellular matrix. Proc. Natl. Acad. Sci. U.S.A. 99, 2714–2719.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Bergsma, D.J., Eder, C., Gross, M., Kersten, H., Sylvester, D., Appelbaum, E., Cusimano, D., Livi, G.P., McLaughlin, M.M., Kasyan, K., Porter, T.G., Silverman, C., Dunnington, D., Hand, A., Prichett, W.P., Bossard, M.J., Brandt, M., Levy, M.A., 1991. The cyclophilin multigene family of peptidyl-prolyl isomerases: characterization of three separate human isoforms. J. Biol. Chem. 266, 23204–23214.

Billich, A., Hammerschmid, F., Peichl, P., Wenger, R., Zenke, G., Quesniaux, V., Rosenwirth, B., 1995. Mode of action of SDZ NIM 811, a nonimmunosuppressive cyclosporin A analog with activity against human immunodeficiency virus (HIV) type 1: Interference with HIV protein–cyclophilin A interactions. J. Virol. 69, 2451–2461.

Brazin, K.N., Mallis, R.J., Fulton, D.B., Andreotti, A.H., 2002. Regulation of the tyrosine kinase Itk by the peptidyl-prolyl isomerase cyclophilin A. Proc. Natl. Acad. Sci. U.S.A. 99, 1899–1904.

Bukrinsky, M.I., 2002. Cyclophilins: unexpected messengers in intercellular communications. Trends Immunol. 23, 323–325.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G., Thompson, J.D., 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res. 31, 3497–3500.

Colgan, J., Asmal, M., Neagu, M., Yu, B., Schneidkraut, J., Lee, Y., Sokolskaja, E., Andreotti, A., Luban, J., 2004. Cyclophilin A regulates TCR signal strength in $CD4^+$ T cells via a proline-directed conformational switch in Itk. Immunity 21, 189–201.

Ewing, B., Green, P., 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8, 186–194.

Ewing, B., Hillier, L., Wendl, M.C., Green, P., 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 8, 175–185.

Frohman, M.A., Dush, M.K., Martin, G.R., 1988. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc. Natl. Acad. Sci. U.S.A. 85, 8998–9002.

Galat, A., 1999. Variations of sequences and amino acid compositions of proteins that sustain their biological functions: an analysis of the cyclophilin family of proteins. Arch. Biochem. Biophys. 371, 149–162.

Grabe, N., 2002. AliBaba2: context specific identification of transcription factor binding site. *In Silico* Biol. 2, S1–S11.

He, Z., Li, L., Luan, S., 2004. Immunophilins and parvulins. Superfamily of peptidyl-prolyl isomerases in Arabidopsis. Plant Physiol. 134, 1248–1267.

Heitman, J., Movva, N.R., Hall, M.N., 1992. Proline isomerases at the crossroads of protein folding, signal transduction, and immunosuppression. New Biol. 4, 448–460.

Helfman, G.S., Collette, B.B., Facey, D.E., 1997. The Diversity of Fishes. Blackwell Science, Malden, MA.

Ivery, M.T., 2000. Immunophilins: switched on protein binding domains? Med. Res. Rev. 20, 452–484.

Jenkins, J.A., Klesius, P.H., 1998. Elicitation of macrophages from the peritoneal cavity of channel catfish. J. Aquat. Anim. Health 10, 69–74.

Kawano, H., Kono, T., Watanuki, H., Savan, R., Sakai, M., 2003. Analysis of genes expressed in head kidney of common carp *Cyprinus carpio* L. treated with cortisol. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 136, 875–886.

Ke, H.M., Zydowsky, L.D., Liu, J., Walsh, C.T., 1991. Crystal structure of recombinant human T-cell cyclophilin A at 2.5 Å resolution. Proc. Natl. Acad. Sci. U.S.A. 88, 9483–9487.

Kim, J.-O., Nau, M.M., Allikian, K.A., Makela, T.P., Alitalo, K., Johnson, B.E., Kelley, M.J., 1998. Co-amplification of a novel cyclophilin-like gene (PPIE) with L-myc in small cell lung cancer cell lines. Oncogene 17, 1019–1026.

Koletsky, A.J., Harding, M.W., Handschumacher, R.E., 1986. Cyclophilin: distribution and variant properties in normal and neoplastic tissues. J. Immunol. 137, 1054–1059.

Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefs Bioinform. 5, 150–163.

Lee, S.P., Hwang, Y.S., Kim, Y.J., Kwon, K.S., Kim, H.J., Kim, K., Chae, H.Z., 2001. Cyclophilin A binds to peroxiredoxins and activates its peroxidase activity. J. Biol. Chem. 276, 29826–29832.

Li, S., Chou, H.-H., 2004. LUCY (2): an interactive DNA sequence quality trimming and vector removal tool. Bioinformatics 20, 2865–2866.

Luban, J., 2007. Cyclophilin A, TRIM5, and resistance to human immunodeficiency virus type 1 infection. J. Virol. 81, 1054–1061.

Maruyama, T., Furuani, M., 2000. Archael peptidyl-prolyl *cis/trans* isomerases (PPIases). Front. Biosci. 5, 821–836.

Mi, H., Kops, O., Zimmermann, E., Jaschke, A., Tropschug, M., 1996. A nuclear RNA-binding cyclophilin in human T cells. FEBS Lett. 398, 201–205.

Min, L., Fulton, D.B., Andreotti, A.H., 2005. A case study of proline isomerization in cell signaling. Front. Biosci. 10, 385–397.

Nickum, J.G., Bart Jr., H.L., Bowser, P.R., Greer, I.E., Hubbs, C., Jenkins, J.A., MacMillan, J.R., Rachlin, J.W., Rose, J.D., Sorensen, P.W., Tomasso, J.R., 2004. Guidelines for the Use of Fishes in Research. American Fisheries Society, Bethesda, Maryland.

Obata, Y., Yamamoto, K., Miyazaki, M., Shimotohno, K., Kohno, S., Matsuyama, T., 2005. Role of cyclophilin B in activation of interferon regulatory factor-3. J. Biol. Chem. 280, 18355–18360.

Pemberton, T.J., 2006. Identification and comparative analysis of sixteen fungal peptidyl-prolyl *cis/trans* isomerase repertoires. BMC Genomics 7, 244.

Price, E.R., Zydowsky, L.D., Jin, M.J., Baker, C.H., McKeon, F.D., Walsh, C.T., 1991. Human cyclophilin B: A second cyclophilin gene encodes a peptidyl-prolyl isomerase with a signal sequence. Proc. Natl. Acad. Sci. U.S.A. 88, 1903–1907.

Rice, P., Longden, I., Bleasby, A., 2000. EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet. 16, 276–277.

Ryffel, B., Woerly, G., Greiner, B., Haendler, B., Mihatsch, M.J., Foxwell, B.M., 1991. Distribution of the cyclosporine binding protein cyclophilin in human tissues. Immunology 72, 399–404.

Schmid, F.X., 2001. Prolyl isomerases. Adv. Protein Chem. 59, 243–282.

Tu, H., Yang, W., Jiang, X., Chen, H., Xiong, Q., Wei, J., Xu, A., 2003. Cloning, sequence analysis and evolutionary conservation of a full-length cDNA encoding cyclophilin A from red stingray *Dasyatis akajei*. Fish Shellfish Immunol. 15, 359–366.

Wang, P., Heitman, J., 2005. The cyclophilins. Genome Biol. 6, 226.

Watashi, K., Ishii, N., Hijikata, M., Inoue, D., Murata, T., Miyanari, Y., Shimotohno, K., 2005. Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. Mol. Cell 19, 111–122.

Yeh, H.-Y., Shoemaker, C.A., Klesius, P.H., 2006. Molecular cloning and sequencing of hemoglobin-β gene of channel catfish *Ictalurus punctatus* Rafinesque. Fish Physiol. Biochem. 32, 83–92.

Yeh, H.-Y., Klesius, P.H., 2007a. cDNA cloning, characterization and expression analysis of channel catfish (*Ictalurus punctatus* Rafinesque, 1818) peroxiredoxin 6 gene. Fish Physiol. Biochem. 33, 233–239.

Yeh, H.-Y., Klesius, P.H., 2007b. Molecular cloning and expression of channel catfish, *Ictalurus punctatus*, complement membrane attack complex inhibitor CD59. Vet. Immunol. Immunopathol. 120, 246–253.

Yurchenko, V., O'Connor, M., Dai, T., Guo, H., Toole, B., Sherry, B., Bukrinsky, M., 2001. CD147 is a signaling receptor for cyclophilin B. Biochem. Biophys. Res. Commun. 288, 786–788.

Yurchenko, V., Zybarth, G., O'Connor, M., Dai, W.W., Franchin, G., Hao, T., Guo, H., Hung, H.C., Toole, B., Gallay, P., Sherry, B., Bukrinsky, M., 2002. Active site residues of cyclophilin A are crucial for its signaling activity via CD147. J. Biol. Chem. 277, 22959–22965.

Yurchenko, V., Pushkarsky, T., Li, J.H., Dai, W.W., Sherry, B., Bukrinsky, M., 2005. Regulation of CD147 cell surface expression: Involvement of the proline residue in the CD147 transmembrane domain. J. Biol. Chem. 280, 17013–17019.

Yurchenko, V., Constant, S., Bukrinsky, M., 2006. Dealing with the family: CD147 interactions with cyclophilins. Immunology 117, 301–309.

Zander, K., Sherman, M.P., Tessmer, U., Bruns, K., Wray, V., Prechtel, A.T., Schubert, E., Henklein, P., Luban, J., Neidleman, J., Greene, W.C., Schubert, U., 2003. Cyclophilin A interacts with HIV-1 Vpr and is required for its functional expression. J. Biol. Chem. 278, 43202–43213.

Zubiaga, A.M., Belasco, J.G., Greenberg, M.E., 1995. The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. Mol. Cell. Biol. 15, 2219–2230.

Zydowsky, L.D., Etzkorn, F.A., Chang, H.Y., Ferguson, S.B., Stolz, L.A., Ho, S.I., Walsh, C.T., 1992. Active site mutants of human cyclophilin A separate peptidyl-prolyl isomerase activity from cyclosporin A binding and calcineurin inhibition. Protein Sci. 1, 1092–1099.